Supporting information

Comparative toxicity of C_{60} aggregates towards mammalian cells: role of the tetrahydrofuran (THF) decomposition

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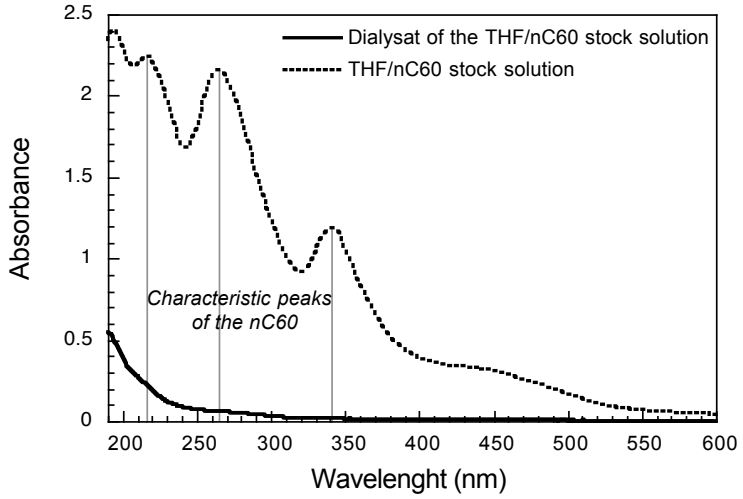

Figure S1. UV-vis graph of the THF/nC_{60} stock solution and the supernatant (liquid phase obtained after dialysis of the stock solution with a cut-off of 4 nm). No signal related to the C_{60} aggregates are observed after dialysis.

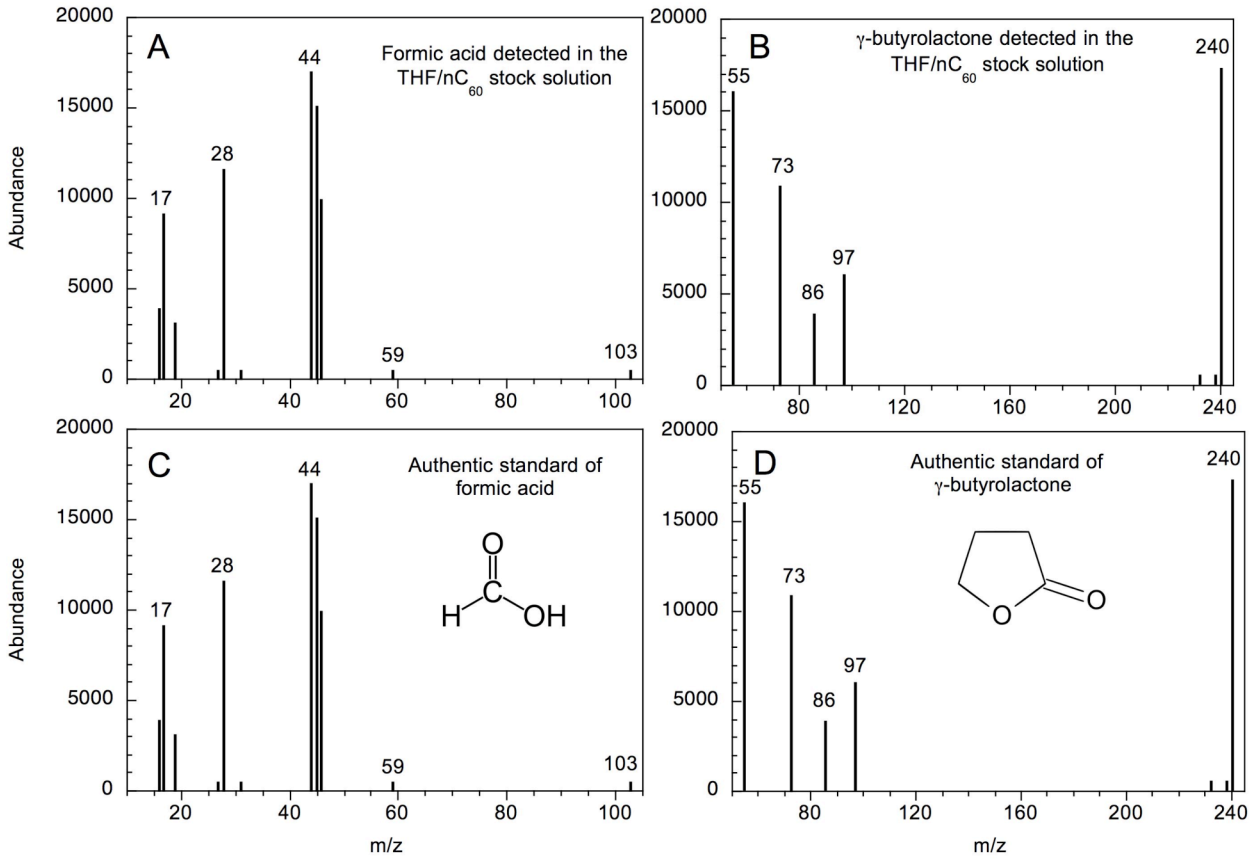

Figure S2. GC-MS spectrum of formic acid (A) and γ-butyrolactone (B) obtained from THF/nC_{60}. (148 ppm extracted in methanol). The spectrum obtained from authentic standard of formic acid (C) and γ-butyrolactone (D).

Concentration of nC_{60}

The concentration of nC_{60} in the aq/nC_{60} and fullerols suspensions was determined by Total Organic Carbon TOC (Shimadzu 5050A, USA) and adjusted to 20 mg/L. As carbon compounds related to THF are present in the THF/nC_{60} suspension, its concentration was determined UV-vis spectrophotometry (Hitachi U2810, USA). The absorbance was measured (200 to 600 nm) on suspensions of known concentration of aq/nC_{60} and unknown concentration of THF/nC_{60}. The curves are superimposed and the normalization factor ($^{THF/nC_{60}}\!/_{aq/nC_{60}}$) gives the relative concentration of nC_{60} in the THF/nC_{60} suspension. The final concentration of nC_{60} is adjusted to 20 mg/L.

ROS measurement

The reduction of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) allows for specific targeting and measurement of superoxide *(1, 2)* when it is combined with the use of superoxide dismutase (SOD), a known quencher of superoxide which cells utilize for protection *(3)*. The reduction of XTT increases the optical density at 470 nm that is estimated using spectrophotometry and used to quantify the amount of superoxide generated. Experiments were prepared by adding 500 μM NADH (a known cellular reductant) to a 5mg/L suspension of THF/nC_{60} or aqu/nC_{60} in DMEM complemented with 5 % of FBS. XTT and SOD were present at a concentration of 100 μM and 25 U/mL respectively. These suspensions were individually mixed in microwell plates using a re-pipetter. UV/Vis absorbance at 470 nm was analyzed using a BMG Labtech FLUOstar Optima Microplate reader (Durham, NC). Differences between the SOD control and the non-SOD reaction were monitored for up to 30 minutes. Absorbance data outside the 95% confidence intervals from each other were considered to be significantly different in terms of superoxide production.

Water chemistry analysis

The content of aq/nC_{60} and fullerols in solution were determined by Total Organic Carbon (Shimadzu 5050A, Columbia, MD, USA). As carbon compounds related to THF are present in the THF/nC_{60} suspension, the concentration is not determined by TOC but using a UV-vis

spectrophotometer U2810 (Hitachi, San Jose, CA). The absorbance is measured as a function of the wavelength (200 to 600 nm) on a suspension of known concentration of aq/nC_{60} and on the THF/nC_{60} suspension of unknown concentration. The two curves are superimposed and the normalization factor (ratio THF/$_{nC60}$ over aq/nC_{60}) gives the relative concentration of nC_{60} in the THF/nC_{60} suspension. After dilution of THF/nC_{60} suspension to match the aq/nC_{60} concentration, another spectra is measured. The ratio is THF/nC_{60} over aq/nC_{60} is measured another time to adjustment of the concentration more accurately. This method has the advantage not to be sensible to a specific wavelength of the spectra (that might change with size distribution) but averages all the wavelengths.

The THF/nC_{60} solutions were analyzed for the presence of THF or sub-products with Ionic Chromatography (IC), Gas Chromatography-Mass Spectrometry (GC/MS) and Gas Chromatography-Flame ionization detector (GC/FID). The IC Dionex DX120 (Sunnyvale, CA) was equipped with a column pack Ionpack AS14A (Dionex) and with an AG14 guard column. The eluent (8mM Na_2CO_3 with 1mM $NaHCO_3$) flows rate is 1 mL/min during 10 min. The IC is equipped with an auto-sampler JASCO 851-AS and an analysis software Ezchrom 6.8 (Pleasanton, CA). For GC/MS, a 2.0 μL aliquot of THF/nC_{60} was injected into the column of a GC/MS-QP5050A series (Shimadzu, Columbia, MD, USA) gas chromatograph mass spectrometer. The helium gas flow rate carrier was 1.0 mL/min through a DB-5MS, 60 m X 0.250 mm, 0.25 μm capillary column. Detection of specific compounds proceeded according to ref. *(4, 5)*.

- *Detection of γ-butyrolactone:* The injector temperature was set to 195°C, the interface temperature was set to 215°C and a split injection ratio was set at 10:1. The column oven temperature was set at an initial value of 40°C, held for 2 minutes, increased to 300°C at a rate of 20°C/min, and held at 300°C for 1 minute. The GC/MS-QP5050A was used in a selected ion monitoring (SIM) acquisition mode. The detector voltage was set to 1.2 kV and the solvent cut time was set to 3 minutes. In the SIM mode, the following ions were monitored: m/z 233, 234, 239, 240, 55, 73, and 97 *(5)*.

The concentration of γ-butyrolactone in a sample of THF/nC_{60} (148 ppm Carbon) was determined using gas chromatography-flame ionization detection (GC/FID). Standard curve were performed using 5 standards from 25 ppm to 500 ppm of γ-butyrolactone in DI water and were extracted in

diethyl ether solvent and run through a GC-17A Shimadzu gas chromatograph. A DBI-30W J&W Scientific fused silica capillary column with a 5.0 μm film thickness was used in the flame ionization process. Assuming that the extraction proceeded in a linear manner corresponding to the original concentrations of each solution, a calibration curve was generated graphing the original concentrations of γ-butyrolactone to the ratio of γ-butyrolactone peak area to the diethyl ether peak area. Using this curve, the ratio of γ-butyrolactone peak from the THF/n-C_{60} sample extraction was used to determine the concentration of γ-butyrolactone. The concentration of γ-butyrolactone was found to be approximately 22 ppm, approximately 8% of the total carbon in the sample.

- *Detection of formic acid:* The injector temperature was set to 250°C, the interface temperature was set to 280°C and a split injection ratio was set at 10:1. The column oven temperature was set at an initial value of 40°C, held for 4.2 minutes, increased to 260°C at a rate of 40°C/min, and held at 260°C for 2 minutes. The GC/MS-QP5050A was used in a selected ion monitoring (SIM) acquisition mode. The detector voltage was set to 1.2 kV and the solvent cut time was set to 3 minutes. In the SIM mode, the following ions were monitored: m/z 27, 28, 31, 44, 45, 46, 31, 59, and 103 *(4)*.

The concentration of formic acid in a sample of THF/nC_{60} (148 ppm Carbon) was determined using a Dionex DX-120 Ion Chromatograph. Samples ranging from 10 ppm to 500 ppm of formic acid (ACS reagent grade from GFS Chemical, Powell, OH) run through the IC apparatus and the peak areas corresponding to each concentration were recorded. From the calibration curve generated graphing the sample concentrations of formic acid against their areas created in the ion chromatography analysis, the concentration of formic acid in the THF/nC_{60} sample was determined. The concentration of formic acid was found to be approximately 8 ppm, approximately 5.4% of the total carbon in the sample.

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